TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA-600/3-83-095		PB83 263665

4. TITLE AND SUBTITLE	5. REPORT DATE
Toxicity and Metabolism Studies with EPA Priority Pollutants and Related Chemicals in Freshwater Organisms	September 1983
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
D.J. Call, L.T. Brooke, N. Ahmad, and J.E. Richter	
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
Center for Lake Superior Environmental Studies University of Wisconsin-Superior Superior, Wisconsin 54880	11. CONTRACT/GRANT NO. 806196, 80020010, 806864
12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
U.S. Environmental Protection Agency Environmental Research Laboratory-Duluth 6201 Congdon Boulevard Duluth, Minnesota 55804	14. SPONSORING AGENCY CODE EPA/600/03

15. SUPPLEMENTARY NOTES

16. ABSTRACT Toxicological studies were conducted in two areas: (1) the toxicity, bioconcentration potential and metabolism of five herbicides in fish; and (2) the toxicity and/or metabolism of priority pollutants and related chemicals in various aquatic organisms.

The test herbicides included alachlor [2-chloro-2',6'-diethyl-N-(methoxymethyl) acetanilide], bromacil [5-bromo-3-sec-butyl-6-methyluracil], dinoseb [2-(sec-butyl)-4,6-dinitrophenol], diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea], and propanil [3,4-dichloropropionanilide]. Acute toxicity (through 192 hr), early life-stage toxicity (58-64 day), and bioconcentration studies were conducted with fathead minnows (Pimephales promelas) in Lake Superior water. Herbicide metabolism was investigated in rainbow trout (Salmo gairdneri) both in vivo and in vitro.

Twenty-two chemicals from the EPA priority pollutant list were studied for their acute and/or chronic toxicity to selected freshwater organisms. These included 1,2-dichloroethane, 1,1,2-trichloroethane, 1,1,2,2-tetrachloroethane, tetrachloroethylene, 1,2-dichlorobenzene, 1,3-dichlorobenzene, 1,4-dichlorobenzene, hexachlorobenzene, hexachlorobutadiene, di-n-butylphthalate, pentachlorophenol, heptachlor, chlordane, toxaphene, arsenic^{+3}, chromium^{+6}, lead^{+2}, mercury^{+2}, nickel^{+2}, silver^{+1}, selenium^{+4}, and cyanide. Freshwater species tested included the fathead minnow, rainbow trout, bluegill sunfish (Lepomis macrochirus), flagfish (Jordanella floridae), Daphnia magna, scud (Gammarus pseudolimnaeus), midge (Tanytarsus dissimilis) and green alga (Selenastrum capricornutum). Toxicity tests were also conducted with pentachloroethane, hexachloroethane, 1,2,4-trichlorobenzene, pentachlorobenzene, methanol and dimethylformamide. The uptake by fish of di-n-butylphthalate from water, its metabolism and elimination were investigated. Comparative metabolism of 1,1,2-trichloroethane, chlorobenzene, 1,1,2-trichloroethylene, chloroform, and carbon tetrachloride was studied in rainbow trout and Daphnia.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	
RELEASE TO PUBLIC	UNCLASSIFIED	
	20. SECURITY CLASS (This page) UNCLASSIFIED	

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

FOREWORD

The Environmental Research Laboratory-Duluth is concerned with effects of chemical pollutants upon aquatic life. Many chemicals are presently in use without adequate knowledge of their effects on aquatic life, and new chemicals are continuously being developed and marketed.

This report contains information on thirty-two chemicals and their effects on freshwater life. Included are values for acute toxicity, chronic toxicity, and metabolism of these chemicals with various species of organisms. These values can be used to provide guidance for the protection of aquatic life.

Norbert Jaworski, Ph. D.
Director
Environmental Research Laboratory
Duluth, MN

DUP040012538

ABSTRACT

Twenty-two chemicals from the EPA priority pollutant list were studied for their acute and/or chronic toxicity to selected freshwater organisms. These included 1,2-dichloroethane, 1,1,2-trichloroethane, 1,1,2,2-tetrachloroethane, tetrachloroethylene, 1,2-dichlorobenzene, 1,3-dichlorobenzene, 1,4-dichlorobenzene, hexachlorobenzene, hexachlorobutadiene, di-n-butylphthalate, pentachlorophenol, heptachlor, chlordane, toxaphene, arsenic^{+3}, chromium^{+6}, lead^{+2}, mercury^{+2}, nickel^{+2}, silver^{+1}, selenium^{+4}, and cyanide. Freshwater species tested included the fathead minnow (Pimephales promelas), rainbow trout (Salmo gairdneri), bluegill sunfish (Lepomis macrochirus), flagfish (Jordanella floridae), water flea (Daphnia magna), scud (Gammarus pseudolimnaeus), midge (Tanytarsus dissimilis), and green alga (Selenastrum capricornutum).

Toxicity tests were also conducted with pentachloroethane, hexachloroethane, 1,2,4-trichlorobenzene, pentachlorobenzene, dimethylformamide and methanol. Di-n-butylphthalate uptake from water, elimination and metabolism by fish was studied. A comparison was made of the metabolism and binding of carbon tetrachloride, chloroform, 1,1,2-trichloroethane, 1,1,2-trichloroethylene and monochlorobenzene by microsomal fractions of rainbow trout livers and of daphnid whole bodies.

DUP040012539

CONTENTS

DUP040012540

DUP040012541

DUP040012542

FIGURES

DUP040012543

TABLES

DUP040012544

DUP040012545

DUP040012546

ACKNOWLEDGEMENTS

We would like to thank our Project Officer, John Teasley, from the Environmental Research Laboratory-Duluth, MN (ERL-D), U.S. Environmental Protection Agency for his cooperation in this study. We are appreciative of assistance and advice from the following ERL-D staff members: William Brungs, Steven Broderius, Charles Stephan, John Poldoski, Roll Syrett, Larry Herman, Gary Phipps, Gary Holcombe, Anthony Carlson, James Fiandt, and Carolanne Curtis. Glenn Endicott and the facilities staff of ERL-D were very helpful. We gratefully recognize the assistance of the following University of Wisconsin-Superior technical staff members: Michael Knuth, Steven Poirier, Catherine Moriarity, Cheryl Anderson, Pamela Shubat, James Huot, Ann Lima, Marilynn Hoglund, Dean Hammermeister, Tom Markee, Taryl Felhaber and Debra Svejskovsky. We thank representatives from Monsanto Corporation for supplying technical grade and radiolabeled di-n-butylphthalate for our studies. We gratefully acknowledge the work of our secretary, Joyce Barnes, in the preparation of this report.

DUP040012547

SECTION I

INTRODUCTION

A 1978 court settlement referred to as the "EPA Consent Decree" between EPA and several environmentally concerned organizations as plaintiffs resulted in the publication of a list of toxic pollutants for which effluent limitations and guidelines were to be developed (Keith and Telliard, 1979). This list of "priority pollutants" initially consisted of 65 chemicals (or groups of chemicals), and was later expanded to 129 entries. EPA was charged with the responsibility of determining the hazard potentials of these "priority pollutants" to aquatic life and human health.

In a formal Cooperative Agreement with EPA, the University of Wisconsin-Superior contracted to perform toxicity tests with selected "priority pollutants" utilizing various species of freshwater organisms in an effort to provide some of the data necessary for the development of water quality criteria statements for the protection of freshwater aquatic life. Toxicity tests were also conducted with several haloalkanes and halobenzenes closely related to "priority pollutants" and with methanol and dimethylformamide which are sometimes used as carrier solvents in toxicity tests.

Studies with mammalian systems have suggested that carbon tetrachloride, chloroform and other chlorinated alkanes are converted to toxic metabolites by the microsomal mixed function oxidase system of the liver (Docks and Krishna, 1976; Watanabe et al., 1978). However, information is limited concerning the

DUP040012548

metabolic disposition and protein binding of such compounds in fish and aquatic food chain organisms. Therefore, one aspect of this study was to investigate the comparative metabolism and protein binding potential of carbon tetrachloride, chloroform, 1,1,2-trichloroethylene, 1,1,2-trichloroethane and monochlorobenzene by microsomal fractions of rainbow trout (Salmo gairdneri) liver and the water flea (Daphnia magna).

DUP040012549

SECTION II

CONCLUSIONS

Acute toxicity tests were conducted with fathead minnows (Pimephales promelas), rainbow trout (Salmo gairdneri), bluegill sunfish (Lepomis macrochirus), flagfish (Jordanella floridae), water fleas (Daphnia magna), scuds (Gammarus pseudolimnaeus), midge larvae (Tanytarsus dissimilis Johannsen 1937), and green algae (Selenastrum capricornutum). Fathead minnows were exposed to arsenic^{+3}, mercury^{+2}, silver^{+1}, dimethylformamide (DMF), and methanol with resulting estimated 96 hr LC_{50} values of 14.2, 0.150, 0.0107, 10,700, and 28,100 mg·L^{-1}, respectively. Rainbow trout were exposed to hexachloroethane, tetrachloroethylene, tetrachloroethylene with DMF as a carrier solvent, DMF, 1,2-dichlorobenzene, 1,4-dichlorobenzene, 1,2,4-trichlorobenzene, pentachlorobenzene with DMF, hexachlorobenzene with DMF, hexachlorobutadiene, and methanol with resultant 96 hr LC_{50} estimates of 0.94, 4.99, 5.84, 10,000, 1.58, 1.12, 1.53, >0.71, >0.0809, 0.320, and 20,000 mg·L^{-1}, respectively.

Bluegill sunfish were exposed to hexachlorobutadiene, hexachlorobenzene with DMF, DMF, and methanol with resultant 96 hr LC_{50} estimates of 0.324, >0.0784, 7,100, and 15,500 mg·L^{-1}, respectively. Flagfish were exposed to arsenic^{+3} and silver^{+1} with resultant 96 hr LC_{50} estimates of 14.4 and 0.0092 mg·L^{-1}, respectively.

Water fleas were exposed to hexachloroethane, pentachloroethane, 1,1,2,2-tetrachloroethane, 1,1,2-trichloroethane, 1,2-dichloroethane, 1,3-dichloro-

DUP040012550

benzene, 1,2,4-trichlorobenzene, tetrachloroethylene, di-n-butylphthalate, DMF, chlordane and nickel^{+2} with resultant unfed 48 hr LC_{50} estimates of 2.90, 7.32, 62.1, 186, 268, 7.43, 2.09, 18.1, 3.70, 14,530, 0.035, and 0.915 $mg \cdot L^{-1}$, respectively. LC_{50} estimates were also made for most of the same compounds in exposures where the organisms were fed. EC_{50} estimates were made for fed and unfed exposures with the chlorinated compounds and with arsenic^{+3}.

Scuds were exposed to pentachlorophenol, arsenic^{+3}, silver^{+1}, lead^{+2}, and chromium^{+6} with resultant 96 hr LC_{50} estimates of 280, 875, 4.49, 140, 67.1 and 94.1 $\mu g \cdot L^{-1}$, respectively. Midge larvae were exposed to hexachloroethane, tetrachloroethylene, 1,2-dichlorobenzene, 1,4-dichlorobenzene, hexachlorobenzene with DMF, pentachlorophenol, DMF, chromium^{+6}, lead^{+2}, silver^{+1}, selenium^{+4}, and cyanide with resultant 48 hr LC_{50} estimates of 5.85, 30.8, 12.0, 13.0, >0.0581, 46.0, 36,000, 57.3, 224, 3.17, 42.5, and 2.36 as HCN or 2.49 as CN^- $mg \cdot L^{-1}$, respectively.

Green algae were exposed to toxaphene and heptachlor for 96 hr with resultant EC_{50} estimates (50% reduction of growth) of 0.38 $mg \cdot L^{-1}$ for toxaphene and 38.1 and 28.2 $\mu g \cdot L^{-1}$ for two tests with heptachlor.

Chronic and subchronic toxicity tests were conducted using water fleas, fathead minnows, and flagfish. Water fleas were exposed to 1,1,2,2-tetrachloroethane, 1,1,2-trichloroethane, 1,2-dichloroethane, 1,2,4-trichlorobenzene, 1,3-dichlorobenzene, 1,2,4-trichlorobenzene, 1,3-dichlorobenzene, tetrachloroethylene, and arsenic^{+3} for 28 days with significant ($p \leq 0.05$ or $p \leq 0.01$) reductions in production of young at concentrations at or above 14.4, 41.8, 20.7, 0.694, 1.45, 1.11, and 1.32 $mg \cdot L^{-1}$, respectively.

Fathead minnows were exposed to arsenic^{+3} for 30 days post-fertilization and mercury^{+2} for 35 days post-fertilization. The "no-effect" concentration

DUP040012551

for arsenic^{+3} was between 2.1 and 4.3 mg·L^{-1} based upon significant ($p \leq 0.01$) reductions in wet weight and body length. Mercury^{+2} exposures resulted in significant ($p \leq 0.01$) reductions in wet weight and length at all exposure concentrations. A "no-effect" concentration for mercury^{+2} was less than the lowest exposure concentration of 0.23 µg·L^{-1}. Flagfish were exposed to arsenic^{+3} for 30 days post-fertilization with a resultant "no-effect" concentration between 2.13 and 4.12 mg·L^{-1} based upon a reduction in body length.

Uptake, elimination, and metabolism of di-n-butylphthalate was studied with fathead minnows. A steady-state level of ^{14}C equivalents of di-n-butylphthalate was attained within 4 hr in the whole-body. Bioconcentration factors in ^{14}C equivalents of di-n-butylphthalate were 2,068 and 2,125 for the two measured exposure concentrations. Estimated bioconcentration factors for parent di-n-butylphthalate were 570 and 590 for the two tests based upon a mean value of 27.6% unmetabolized compound over an 11 day exposure.

Seven metabolites of di-n-butylphthalate were separated by thin-layer chromatography after three days of exposure. The only metabolite identified was phthalic acid.

Binding of di-n-butylphthalate to proteins was studied using rainbow trout liver microsomes and water flea post-mitochrondrial supernatant (PMS). Irreversible binding to proteins occurred with 9% of the compound bound to the rainbow trout liver microsomes in 2hr and <1% irreversibly bound to water flea PMS in 1 hr.

Microsomal metabolism and binding of carbon tetrachloride, chloroform, chlorobenzene, 1,1,2-trichloroethylene, and 1,1,2-trichloroethane were studied with rainbow trout liver microsomes and water flea PMS. The compounds were metabolized by both species with rainbow trout appearing to have a greater

DUP040012552

capacity for metabolizing them. The compounds were metabolized by rainbow trout in the following order: chloroform > 1,1,2-trichloroethane, > 1,1,2-trichloroethylene > chlorobenzene > carbon tetrachloride. The compounds were metabolized by water fleas in the following order: chloroform > chlorobenzene > 1,1,2-trichloroethylene > 1,1,2-trichloroethane ≈ carbon tetrachloride.

Mixed function oxidase assays were performed on the microsomal fraction from rainbow trout liver and the water flea PMS fraction. Rainbow trout liver microsomes had 0.28 and 0.19 $nM \cdot mg^{-1}$ of cytochrome P-450 and cytochrome b_5, respectively. The level of NADPH cytochrome c reductase activity was 16 nM of cytochrome c reduced$\cdot min^{-1} \cdot mg^{-1}$ protein. Water flea PMS had 42 nM of cytochrome c reductase activity$\cdot min^{-1} \cdot mg^{-1}$ protein.

DUP040012553

SECTION III

RECOMMENDATIONS

Adequate assessment of a particular chemical's toxicity would be enhanced by exposing the chemical to many species of aquatic organisms representing the taxa likely to be impacted in the environment. Particular effort should be expended with species shown to be generally sensitive to the class of compound of immediate interest. Organisms such as Tanytarsus dissimilis (midge) with consistently high tolerances to a broad range of chemical classes should receive minimal effort.

Physical properties (i.e. hardness, pH, temperature) of the laboratory test water need to be carefully monitored, and several waters with different natural chemical characteristics used for exposures to assess the impacts of these parameters. The effects that water hardness and organic ligands have upon toxicity of some metals are known but not completely understood. Studies should be conducted to elucidate the relationships between chemical characteristics of natural waters and pollutant toxicity.

A better understanding is needed of compound metabolism and enzyme induction in aquatic organisms. Additional research on the capabilities of animals from various taxonomic groups to metabolize foreign chemicals would be valuable.

DUP040012554

SECTION IV

METHODS

Water Supply and Environmental Control

Water for the toxicity tests was either directly from Lake Superior or was dechlorinated city water from Superior, WI. (Superior, WI derives its water from shallow wells beneath Lake Superior.) Several chemical parameters (dissolved oxygen, pH, hardness, acidity, and alkalinity) were monitored during the fish and scud toxicity tests by standard analytical methods (American Public Health Association, 1975). A portion of the water was heated before being distributed to the test systems. Lighting for the toxicity tests was artificial, supplied by fluorescent bulbs centered above the exposure chambers.

Test Organisms

Fathead minnow (Pimephales promelas) brood fish were received from stock maintained by the Environmental Research Laboratory-Duluth, MN, U.S. EPA. Brood fish were maintained at 25 C, and were fed twice daily a diet of frozen adult brine shrimp.

Asbestos pipe (12.5 cm O.D.) cut in half, longitudinally, was used as the spawning substrate. The spawning substrates (tiles) were checked daily for egg deposition. Eggs were removed from the tiles the same day ($\leq$ 24 hr) that spawning occurred when used in early life-stage tests. Eggs were allowed to remain on the tiles and were cared for by brood stock males until 50% or more were "eyed up" for later use in acute toxicity tests. Tiles with "eyed up"

DUP040012555

in Douglas County, WI. They were acclimated and reared in 56 L glass chambers with continuously flowing Lake Superior water at 20 C. The organisms were fed leaves of various deciduous species of trees native to St. Louis County, MN, that had been soaked in lake water for at least one month. Reproduction of the scuds occurred in the rearing chambers. At the start of a toxicity test organisms were selected based upon size uniformity with no attempt to determine age.

A midge (Tanytarsus dissimilis Johannson 1937) culture was maintained from stock organisms received from the Environmental Research Laboratory-Duluth, MN, U.S. EPA. The colony was maintained at a water temperature of approximately 20 C on a diet of Cerophyl® and trout pellets as described by Anderson et al.

from 6.8 to 10.6 volume additions per day. Water temperature was maintained at approximately 12 C. Ten fish were tested per chamber. Fish were of the following sizes for the individual tests: tetrachloroethylene - 6.1 ± 1.0 cm, 3.2 ± 1.5 g (n=19); tetrachloroethylene with dimethylformamide (DMF) carrier-solvent 7.3 ± 1.0 cm, 5.86 ± 2.45 g (n=19); 1,2-dichlorobenzene - 5.6 ± 0.8 cm, 2.69 ± 1.24 g (n=10); 1,4-dichlorobenzene - 5.3 ± 0.6 cm, 2.1 ± 1.0 g (n=20); hexachlorobutadiene - 5.6 ± 0.6 cm, 2.6 ± 0.9 g (n=19); hexachloroethane - 6.6 ± 1.0 cm, 4.3 ± 1.8 g (n=20); 1,2,4-trichlorobenzene - 4.7 ± 0.4 cm, 1.6 ± 0.4 g (n=20).

Water quality parameters were routinely measured (Appendix A, Table A-1). Toxicant concentrations were measured daily as described with fathead minnows (Appendix B, Table B-1).

Penta- and hexachlorobenzene acute tests were conducted in a different type of flow-through system due to their limited water solubilities. Pentachlorobenzene was tested with dimethylformamide (DMF) as a carrier solvent. Penta-chlorobenzene/DMF stock stolutions of known concentrations were pumped into test chambers with fluid metering pumps. The control chambers

DUP040012556

from 6.8 to 10.6 volume additions per day. Water temperature was maintained at approximately 12 C. Ten fish were tested per chamber. Fish were of the following sizes for the individual tests: tetrachloroethylene - 6.1 ± 1.0 cm, 3.2 ± 1.5 g (n=19); tetrachloroethylene with dimethylformamide (DMF) carrier-solvent 7.3 ± 1.0 cm, 5.86 ± 2.45 g (n=19); 1,2-dichlorobenzene - 5.6 ± 0.8 cm, 2.69 ± 1.24 g (n=10); 1,4-dichlorobenzene - 5.3 ± 0.6 cm, 2.1 ± 1.0 g (n=20); hexachlorobutadiene - 5.6 ± 0.6 cm, 2.6 ± 0.9 g (n=19); hexachloroethane - 6.6 ± 1.0 cm, 4.3 ± 1.8 g (n=20); 1,2,4-trichlorobenzene - 4.7 ± 0.4 cm, 1.6 ± 0.4 g (n=20).

Water quality parameters were routinely measured (Appendix A, Table A-1). Toxicant concentrations were measured daily as described with fathead minnows (Appendix B, Table B-1).

Penta- and hexachlorobenzene acute tests were conducted in a different type of flow-through system due to their limited water solubilities. Pentachlorobenzene was tested with dimethylformamide (DMF) as a carrier solvent. Pentachlorobenzene/DMF stock stolutions of known concentrations were pumped into 5 test chambers with fluid metering pumps. The control chambers received DMF only. DMF concentrations were nominally equal and averaged 395 $mg \cdot L^{-1}$ between exposure chambers. Test chambers were 30 x 60 x 30 cm, and contained 27 L of water (depth of 15 cm). Pumps were set to deliver every time the system cycled and delivered 1 L of Lake Superior water to each chamber. The cycle time of the system averaged 16.5 min, providing 3.2 volume additions of water per day. The mean water temperature was 12.7 C.

The test was run with 10 fish [mean standard length, 6.9 ± 1.2 cm; mean weight, 5.2 ± 2.5 g (n=20)] per chamber. Replicates were separated in time by 11 days. Since there were insufficient deaths at 96 hr to calculate an LC_{50}

DUP040012557

concentration, the exposures were continued through 144 hr.

Hexachlorobenzene was tested in the system as described for pentachlorobenzene, also with DMF as a carrier-solvent. Two toxicant concentrations plus a control in duplicate were used. The lower concentration was at or near the solubility limit and the higher concentration exceeded water solubility. DMF concentrations were nominally equal in all chambers, and averaged 932 $mg \cdot L^{-1}$. Test duration was 96 hr at a mean water temperature of 11.2 C. The cycle time of the system averaged 8.25 min, providing 6.5 volume additions of water per day. Ten fish per tank were tested. Mean fish length and weight values were 3.3 ± 0.3 cm and 0.5 ± 0.1 g (n=20), respectively.

Toxicant concentrations were measured daily (Appendix B, Table B-1). Water quality parameters for penta- and hexachlorobenzene tests are presented in Appendix A, Table A-1.

Bluegill Sunfish - Bluegill sunfish were used as test organisms in acute tests with hexachlorobutadiene and hexachlorobenzene. In the test with hexachlorobutadiene, a proportional diluter system was used. Chamber dimensions were 51 x 15 x 15.5 cm, with a 10 cm water depth for a volume of 7.7 L. The diluter cycle time was 9.1 min, providing 10.3 volume additions per day. Ten fish were exposed per chamber. Mean standard length of the fish was 3.9 ± 0.5 cm, and mean weight was 1.5 ± 0.6 g (n=20). Water temperature was maintained at 25.2 C.

Hexachlorobenzene was tested with bluegill sunfish in the system as described for hexachlorobenzene and rainbow trout, with two toxicant concentrations and a control in duplicate. DMF was used as a carrier-solvent, and averaged 884 $mg \cdot L^{-1}$ in all chambers. The delivery system cycled every 9.7 min, providing 5.5 volume additions of water daily. The test was conducted at a

DUP040012558

mean water temperature of 23.3 C. Ten fish were tested per chamber at a mean standard length of 2.9 ± 0.4 cm and a mean weight of 0.4 ± 0.1 g (n=10). Water quality parameters and toxicant concentrations are presented in Appendices A and B, Tables A-1 and B-1, respectively.

Flagfish. Flagfish were used in acute tests with arsenic^{+3} and silver^{+1}. Flagfish 34 days of age (standard length, 13 ± 2.0 mm; weight, 0.058 ± 0.027 g, n=20) were exposed to arsenic^{+3} in a proportional diluter system using dechlorinated city water. They were tested simultaneously with fathead minnows in glass exposure chambers (20.5 x 30 x 25 cm) divided with screen into two sections. Flagfish sections contained an average of 2.1 L of water. Twenty fish per chamber were tested. The diluter cycle time of 19 min provided 6.9 volume additions of water per day. The test was conducted at a mean water temperature of 25.8 C.

Flagfish 30 days of age (standard length, 12.7 ± 1.6 mm; weight, 0.044 ± 0.021 g, n=30) were exposed to silver^{+1} in a proportional diluter system using Lake Superior water. They were tested simultaneously with fathead minnows in glass chambers (15 x 30 x 25 cm) divided by screen into two sections. Fifteen fish per chamber were tested. The diluter cycle time of 16 min provided about 8 volume additions of water per day. Mean test water temperature was 24.7 C. Water quality parameters and toxicant concentrations were measured routinely throughout the tests (Appendices A and B, Tables A-1 and B-1, respectively).

Daphnia magna - Daphnia magna was used as the test species in static acute tests conducted with 1,2-dichloroethane, 1,1,2-trichloroethane, 1,1,2,2-tetrachloroethane, pentachloroethane, hexachloroethane, tetrachloroethylene, 1,3-dichlorobenzene, 1,2,4-trichlorobenzene, di-n-butylphthalate, chlordane, nickel^{+2}, and arsenic^{+3}. Acute tests with chlorinated ethanes, ethylene, and

DUP040012559

benzene were conducted using first instar (≤ 24 hr old) daphnids. Adult daphnids were originally obtained from the laboratory stock reared at the Environmental Research Laboratory-Duluth, MN. Both stock and test animals were maintained in a constant temperature water bath (20 ± 1 C).

A combination of Gro-Lux and Duro-Test (Optima FS) fluorescent bulbs provided 32 ft-candles of light at the air-water interface, and were set for a 16L:8D photoperiod coupled with a 15 min transition period between light and dark phases. All culturing and testing was done with Lake Superior water which was filtered (5 μm) and aerated. All chemical stock solutions were prepared by saturating lake water with the test chemical on a stirring plate.

Acute tests were conducted according to the "Proposed Standard Practice of Conducting Basic Acute Tests with Fishes, Macroinvertebrates, and Amphibians - Draft No. 8". Test containers were 200 mL erlenmeyer flasks filled to 200 or 160 mL for tests in which the daphnids were unfed or fed, respectively. The flasks were tightly stoppered with foil wrapped neoprene stoppers. Food concentration was 20 $mg \cdot L^{-1}$. The measure of acute toxicity was the 48 hr median effective concentration (48 hr EC_{50}) based upon complete immobilization and the 48 hr median lethal concentration (48 hr LC_{50}) based upon death as determined by cessation of heart beat and gut movement. Both were determined using a 30X dissection scope.

Toxicant concentrations were measured during each test (Appendix B, Table B-1). Water quality parameters are presented in Appendix A, Table A-2.

The Daphnia acute test with di-n-butylphthalate was a renewed static test using Lake Superior water, in which toxicant solutions were renewed daily, and test organisms were transferred daily by wide-mouthed pipettes into the new solutions. Exposures were conducted in 200 mL erlenmeyer flasks containing

DUP040012560

150 mL of solution. Five first instar (<24 hr old) daphnids per duplicate flask were tested. The test organisms were obtained from the Environmental Research Laboratory-Duluth, MN (U.S. EPA) stock culture. Culture and test organisms were maintained at 20 ± 2 C. A 16L:8D photoperiod was used with a light intensity of 45 ft-candles. Flasks were observed daily for mortalities. Water quality parameters and toxicant concentrations are presented in Appendices A and B, Tables A-2 and B-1, respectively.

The Daphnia acute static test with chlordane was conducted with Lake Superior water (first instar, <24 hr old from the Environmental Research Laboratory-Duluth, MN stock cultures). Ten organisms were placed into solutions in 200 mL erlenmeyer flasks. Five concentrations and a control were tested, in duplicate. The flasks were kept in a 21 C water bath, and a 16L:8D photoperiod was used. Flasks were observed daily for mortalities. Water quality parameters and toxicant concentrations were measured (Appendices A and B, Tables A-2 and B-1, respectively).

The Daphnia acute test with nickel^{+2} was conducted in Lake Superior water, using 10 organisms (first instar <24 hr old, from the Environmental Research Laboratory-Duluth, MN stock culture) per flask. Five concentrations plus a control in duplicate, were tested in a 20 C water bath. The flasks were 300 mL erlenmeyers containing approximately 250 mL of water. A 16L:8D photoperiod was used, with a light intensity of 60 ft-candles. Water quality parameters and toxicant concentrations are presented in Appendices A and B, Tables A-2 and B-1, respectively. Flasks were observed daily for mortalities.

The Daphnia acute test with arsenic^{+3} was conducted in Lake Superior water using 9-11 organisms (first instar, 24 ± 12 hr old, from the University of Wisconsin-Superior, WI stock culture) per flask. Six concentrations plus a

DUP040012561

control in duplicate were tested. The flasks were maintained at a temperature of 14.8 ± 0.8 C, with a photoperiod of 16L:8D. Acute tests were run with organisms both fed throughout exposure and not fed. Flasks were observed daily for mortalities. Water quality parameters and toxicant concentrations were measured (Appendices A and B, Tables A-2 and B-1, respectively).

Scuds - Gammarus pseudolimnaeus was tested in acute tests conducted with pentachlorophenol, arsenic^{+3}, silver^{+1}, lead^{+2}, and chromium^{+6}. All were flow-through tests conducted in proportional diluter systems (Mount and Brungs, 1967) using Lake Superior water.

In the pentachlorophenol test, 15 organisms ($\bar{x}$ = 0.050 ± 0.016 g) per chamber were tested. Chamber dimensions were 25.5 x 17 x 15 cm, with a water depth of 9 cm, for a volume of 3.9 L. The diluter cycled every 16 min, providing 11.5 volume additions per day. A 16L:8D photoperiod was used, with a light intensity of 20-31 ft-candles. The test was conducted at a water temperature of 17.1 ± 0.5 C. Five exposures plus a control, in duplicate, were used. Organisms were considered dead when movement ceased and they would not respond to prodding. Water quality parameters and toxicant concentrations were monitored throughout the test (Appendices A and B, Tables A-1 and B-1, respectively).

In the test with arsenic^{+3}, 10 organisms (0.3 - 1.1 cm, total length) per chamber were tested. Chamber dimensions were 6.3 x 6.3 x 9.3 cm. These chambers were placed inside larger glass chambers (26 x 17 x 15 cm) containing exposure water 8 cm deep. The inner chambers were constructed of glass on two sides and the bottom. Two sides were made of 202 Nitex® mesh to allow exchange of exposure water from the larger chambers. Exposure water passively entered each chamber containing the test organisms, and toxicant concentrations were identical both inside and outside of the inner chambers. The diluter cycle time averaged

DUP040012562

16 min, providing 12.9 volume additions per day. The photoperiod and light intensity were the same as above. Water temperature was 18.4 ± 0.9 C. Five exposures, plus a control, in duplicate were tested. Water quality parameters and toxicant concentrations are presented in Appendices A and B, Tables A-1 and B-1, respectively.

An acute test with Gammarus and silver^{+1} was conducted under the same conditions as described for the arsenic^{+3} test. Ten organisms (0.3 - 1.1 cm, total length) per chamber were tested. The diluter cycle time was 15.8 min, providing 13.1 volume additions of water per day. The test water temperature was 19.9 ± 0.5 C. Water quality parameters and toxicant concentrations were monitored throughout the test (Appendices A and B, Tables A-1 and B-1, respectively).

An acute test with Gammarus and lead^{+2} was conducted under identical conditions as described for the arsenic^{+3} test. Fifteen organisms (0.053 ± 0.021 g) per chamber were tested at a water temperature of 17.6 ± 0.4 C. Water quality parameters and toxicant concentrations are presented in Appendices A and B, Tables A-1 and B-1, respectively.

Midges - Tanytarsus dissimilis was the test species for acute tests conducted with tetrachloroethylene, 1,2-dichlorobenzene, 1,4-dichlorobenzene, hexachlorobenzene, pentachlorophenol, hexachloroethane, chromium^{+6}, lead^{+2}, silver^{+1}, selenium^{+4}, and cyanide. All tests were conducted using Lake Superior water. Midge exposures were conducted in 8.5 cm diameter glass crystallizing dishes filled to a depth of 2.6 cm (~200 mL volume) with test solutions. Each chamber with a 3 mm glass overflow tube contained lake water plus a small amount of food (ratio of 1L:0.0025 L), along with a fine layer of sand on the bottom. Food was a mixture of Cerophyll® and trout pellets blended with water.

DUP040012563

Ten to 20 midge larvae in their 3rd or 4th instar stage of development (2.0 - 3.5 mm total length) were placed into each dish containing Lake Superior water, plus food, and were allowed to acclimate overnight (or for 48 hr in the silver^{+1} and selenium^{+4} tests) in a 20 C water bath. Hexachlorobenzene and pentachlorophenol tests were acclimated and run at room temperature (22.5 - 25.6 C). A photoperiod of 16L:8D at an intensity of 1.9 ft-candles was maintained throughout the test. In the case of the silver^{+1} test, the light was turned off at 24 hr due to an observable color change (graying) at the higher toxicant concentrations. In the selenium^{+4} test, the midges were exposed to room light only during working hours.

Each dish was examined after 24-48 hrs of acclimation for normal movement and case building. Midges were replaced if they were immobile or were building pupation cases. The water was siphoned off to a depth of 2-3 mm, and toxicant slowly dripped in from a separatory funnel at a rate of approximately 2 mL·min^{-1}. Five toxicant concentrations plus a control, in duplicate, were tested, with the exception of hexachlorobenzene.

Hexachlorobenzene was tested at two concentrations plus a control, in duplicate. The crystallizing dishes contained 150 mL of solution and were covered. Nominal toxicant concentrations were 5.2 and 94.1 µg·L^{-1}. DMF was used as a solvent carrier at a mean concentration of 1086 mg·L^{-1}. The crystallizing dishes were twice siphoned and replaced with fresh hexachlorobenzene/DMF solutions at the beginning of the test in an attempt to maintain nominal concentrations. This test was run at a temperature of 23.9 ± 1.2 C.

Midges were observed on a light table at various time intervals through 48 hr. Effects and deaths were recorded. Death was defined as complete lack of movement when prodded.

DUP040012564

The water was analyzed daily for toxicant concentrations (Appendix B, Table B-1). Water quality parameters were also monitored (Appendix A, Table A-1).

Green Algae - Selenastrum capricornutum was tested in 96 hr toxicity tests using toxaphene and heptachlor. Algal tests were conducted in 125 mL erlenmeyer flasks stoppered with foam plugs on a shaker platform. The flasks were placed in an environmental chamber and incubated under controlled conditions of light and temperature. Light intensity was 400 ft-candles and the test temperature was 24 C.

The nutrient solution concentration was twice the concentration used in the 1978 Selenastrum Bottle Test Procedure (Miller et al., 1978), providing for greater biomass production and more reliable dry weight measurements. Ethanol was used as a carrier-solvent in the toxaphene test, and all test flasks contained a concentration of 0.4% ethanol. No carrier-solvent was used in the heptachlor test.

Nutrient solutions containing toxaphene or heptachlor were inoculated with a 4-5 day-old culture of Selenastrum to yield an initial density of 20,000 cells·mL^{-1} in each flask. Triplicate flasks were inoculated at each exposure level (control plus 5 toxicant concentrations) for biomass determinations after 96 hr of exposure. After 96 hr, individual control and exposure flask solutions containing algae were filtered through pre-weighed 0.45 µm filters, dried, and weighed to determine algal biomass. Mean algal weights at various exposure levels were compared to the mean algal weight of the control group, and expressed as percentage inhibition of growth. The initial toxicant concentration that inhibited growth by 50% (EC_{50}) was determined from interpolation by the trimmed Spearman-Karber method.

DUP040012565

PB83-263665

EPA-600/3-83-095
September 1983

TOXICITY AND METABOLISM STUDIES WITH EPA PRIORITY POLLUTANTS AND RELATED CHEMICALS IN FRESHWATER ORGANISMS

by

Daniel J. Call, Larry T. Brooke, Nasim Ahmad, and
Joseph E. Richter
Center for Lake Superior Environmental Studies
University of Wisconsin-Superior, Superior, WI 54880

12206

U.S. EPA Grant No. R 880020010
U.S. EPA Cooperative Agreement Nos. CR 806864020 & CR 806864030

Project Officer
John I. Teasley
Environmental Research Laboratory - Duluth
Office of Research and Development
U.S. Environmental Protection Agency
Duluth, Minnesota 55804

REPRODUCED BY
U.S. DEPARTMENT OF COMMERCE
NATIONAL TECHNICAL
INFORMATION SERVICE
SPRINGFIELD, VA 22161

DUP040012566